SILVA BALCANICA

Silva Balcanica 24(2): 5–12 (2023)
doi: 10.3897/silvabalcanica.24.e108600

RESEARCH ARTICLE

Epizootic in gypsy moth (*Lymantria dispar*) population in the field protective forest belts of State Hunting Enterprise Balchik in 2022

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Academic editor: Ivailo Markoff | Received 10 June 2023 | Accepted 30 June 2023 | Published 04 July 2023

Citation: Georgiev G., Georgieva M., Mirchev P., Belilov S., Matova M., Ivanov V., Radev R., Kirilova M. 2023. Epizootic in gypsy moth (*Lymantria dispar*) population in the field protective forest belts of State Hunting Enterprise Balchik in 2022. Silva Balcanica 24(2): 5–12. https://doi.org/10.3897/silvabalcanica.24.e108600

Abstract

Biological control of gypsy moth (*Lymantria dispar*) was carried out in 2021 in the field protective forest belts at the territory of the State Hunting Enterprise Balchik by introducing the entomopathogenic fungus *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae). In the same year a mortality rate of 26.1% of the pest population was registered. In 2022 *E. maimaiga* and multiple nuclear polyhedrosis virus of *L dispar* (*Ld*MNPV) caused a population collapse of the pest in the region. Azygospores of *E. maimaiga* were reported in the cadavers of about 5% of middle- and late-instar dead host larvae with *Ld*MNPV polyhedra, which is an indirect indication of initial infection by the fungal pathogen. The epizootic of gypsy moth in the Balchik region is the first recorded case of coinfection by *E. maimaiga* and *Ld*MNPV in Bulgaria.

Keywords

Lymantria dispar, calamity, *Entomophaga maimaiga*, *Ld*MNPV, Bulgaria

Introduction

The gypsy moth (*Lymantria dispar* L., Lepidoptera: Erebidae) is a Palearctic species widely distributed in Europe, Asia and North Africa, which periodically (every 8-12 years) forms calamities and causes defoliation in broad-leaved forests over large

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areas (Georgiev, 2018; McManus, Csóka, 2007; Zúbrik et al., 2016, etc.). In Bulgaria, the pest had caused more than a half of all damage in the forests (Mirchev et al., 2003; Zaemdzhikova et al., 2019).

In the late 1860s, *Lymantria dispar* was accidentally introduced to North America (McManus, McIntyre, 1981). Due to the absence of natural enemies, the pest quickly had spread to the eastern regions of the United States and Canada, causing enormous damage to the forest vegetation (Forbush, Fernald, 1896; Montgomery, Wallner, 1988; Gottschalk, 1990; Ridley et al., 2000). In the newly occupied territories, numerous programs for biological control of the pest via introducing parasitoids and pathogens from different parts of its natural range had been implemented (Reardon, 1981; Coulson, 1981, etc.). Among them, the most effective was the introduction in 1989 of the specific fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper (Entomophthorales: Entomophthoraceae) from Japan (Smitley et al., 1995; Hajek, 1999; Hajek et al., 1995, 2005, etc.). Currently, the pathogen has a major impact on population density of *L. dispar* in the United States (Tobin, Hajek, 2012).

Entomophaga maimaiga was introduced in Bulgaria in 1999 (Pilarska et al., 2000, 2006). The pathogen has been successfully used to suppress a number of calamities of gypsy moth in Bulgaria and countries of the Balkan Peninsula (Georgiev et al., 2013a; Mirchev et al., 2013; Pilarska et al., 2016).

In 2021, *E. maimaiga* was used for biological control of *L. dispar* in the field protective forest belts at the territory of State Hunting Enterprise (SHE) Balchik, where mortality of more than a quarter (26.1%) of the host population was reported (Georgiev et al., 2021).

This article presents the results of studies on the impact of *E. maimaiga* on the gypsy moth population in the field protective forest belts at the territory of SHE Balchik in 2022.

Material and Methods

Entomophaga maimaiga was introduced on 17 March 2021 in ten places within the territory of SHE Balchik through inoculum (dead *L. dispar* larvae) collected during an epizootic in 2014 in the region of State Forest Enterprise Kirkovo (Georgiev et al., 2021). Dead larvae, containing azygospores of *E. maimaiga*, were stored in wooden boxes in the soil at a depth of 20-30 cm. The inoculum mixed with soil was applied around the base of the stems of five trees in each experimental site.

In 2022, three surveys of the *L. dispar* population were carried out – two in the larval stage (on 22 June and 14 July) to determine pathogen infestation, and one (in September) to count the number of egg masses of the new generation and determine the effect of the introduction.

The biological material for research in laboratory conditions was collected from one of the places of introduction (field protective forest belt 2339-b). Microscopic

analyses were performed with an OPTIKA B-1000 light microscope at 125× and 250× magnification, and the photographs were taken with an OPTIKA C-P8 digital camera at 250× magnification. The infection by *E. maimaiga* was determined by the presence of azygospores and conidia, and by *Lymantria dispar* multiple nuclear polyhedrosis virus (*Ld*MNPV) – by polyhedra in dead larvae of the host.

Results

The survey on 22 June 2022 showed that *L. dispar* larvae were in IV-VI instar, concentrated at the base of the stems. Individual specimens died, with typical symptoms of infection by *E. maimaiga* (seized with widely spread legs, hanging upside down) (Fig. 1A) or *Ld*MNPV (with hanging anterior and hindquarters clamped at mid-body, with decomposing and oozing viscera) (Fig. 1B). At the time of examination, the majority of the larvae were alive (Fig. 1C). On 14 July 2022, the gypsy moth larvae were found dead, with leaked internal contents. Chitinous skin sheaths remained on the trees (Fig. 1D), but the majority of larvae fell to the soil at the base of the stems (Fig. 1E).

Light microscope analyses of the dead *L. dispar* larvae showed the presence of a fungal-viral co-infection. Single (Fig. 2A) or a group of azygospores of *E. maimaiga* (Fig. 2B) with a limited concentration of *Ld*MNPV polyhedra were found in the bodies of a small part of the larvae (less than 5%), and in the remaining larvae – a high concentration of *Ld*MNPV polyhedra without azygospores of *E. maimaiga* (Fig. 2C).

The population density of *L. dispar* in autumn 2020 (2.7-15.9 egg masses/tree) is comparable to the population density of the species in 2021 (1.4-10.7 egg masses/tree) (Georgiev et al., 2021).

In September of 2022, only single egg masses of gypsy moth were registered in the field protective forest belts at the territory of SHE Balchik. The population density of the pest was very low (0.03-0.20 egg masses/tree) (Fig. 3).

Discussion

It has been suggested that the low efficiency of the introduction of *E. maimaiga* in 2021 in the field protective forest belts in the SHE Balchik region is most likely related to the lack of rainfall in April and May, which does not allow the development of conidial infection of the pathogen (Georgiev et al., 2021). The lack of a characteristic forest canopy in the field protective forest belts also contributes to lower atmospheric humidity, and therefore lower effectiveness of the fungal pathogen.

The occurrence of infections in insects depends on the combined action of host population density, pathogen and environmental conditions. *E. maimaiga* is known to be effective over a very wide range of population densities of gypsy moth – less

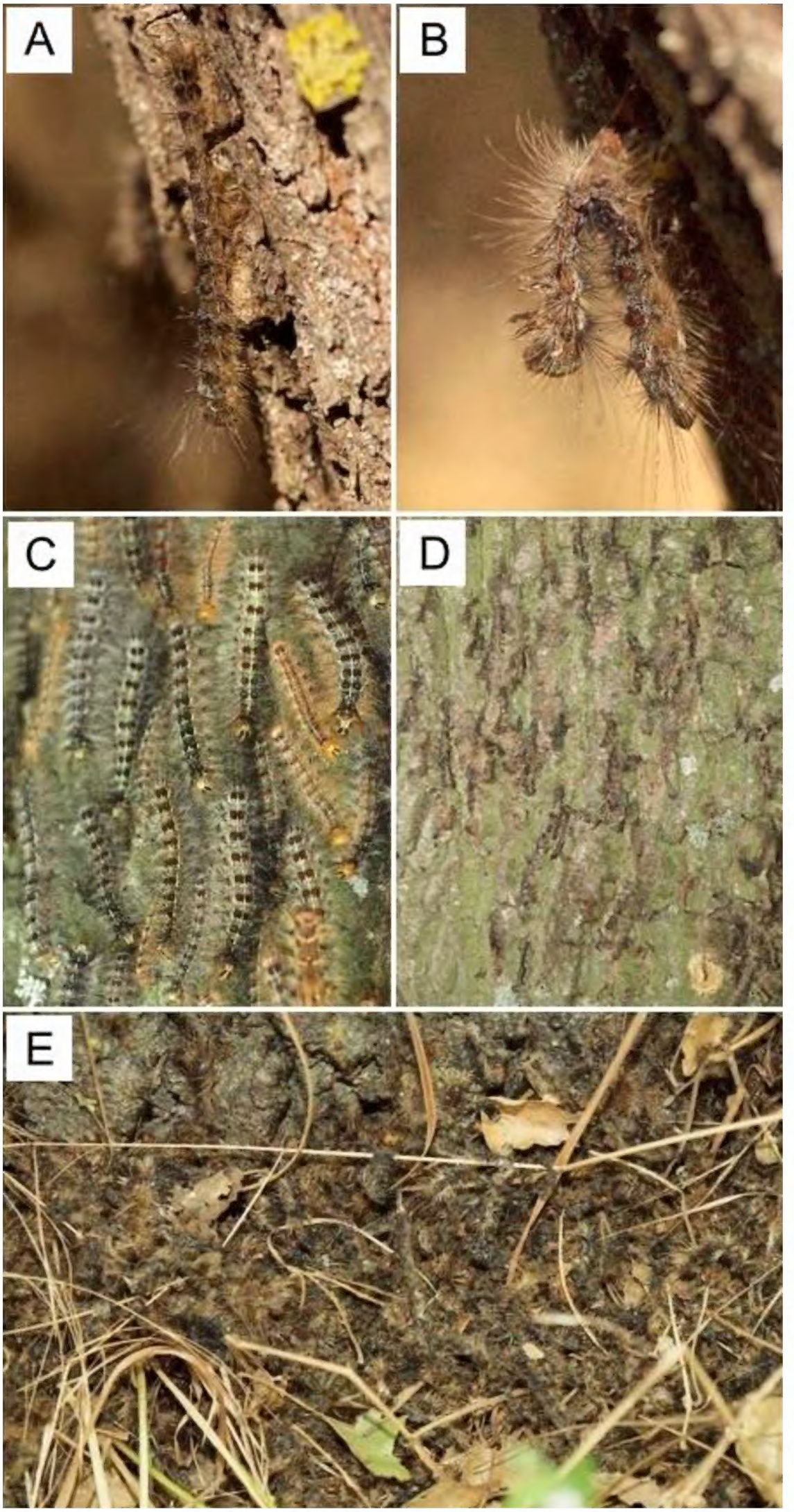

Figure 1. Epizootic of *Lymantria dispar*: A – dead larva infected by *Entomophaga. maimaiga* (22.06.2022); B – dead larva infected by *Ld*MNPV (22.06.2022); C – live larvae at the base of tree stems (22.06.2022); D – remains of dead larvae on tree stems (14.07.2022); E – remains of dead larvae on the soil next to the tree stems (14.07.2022)

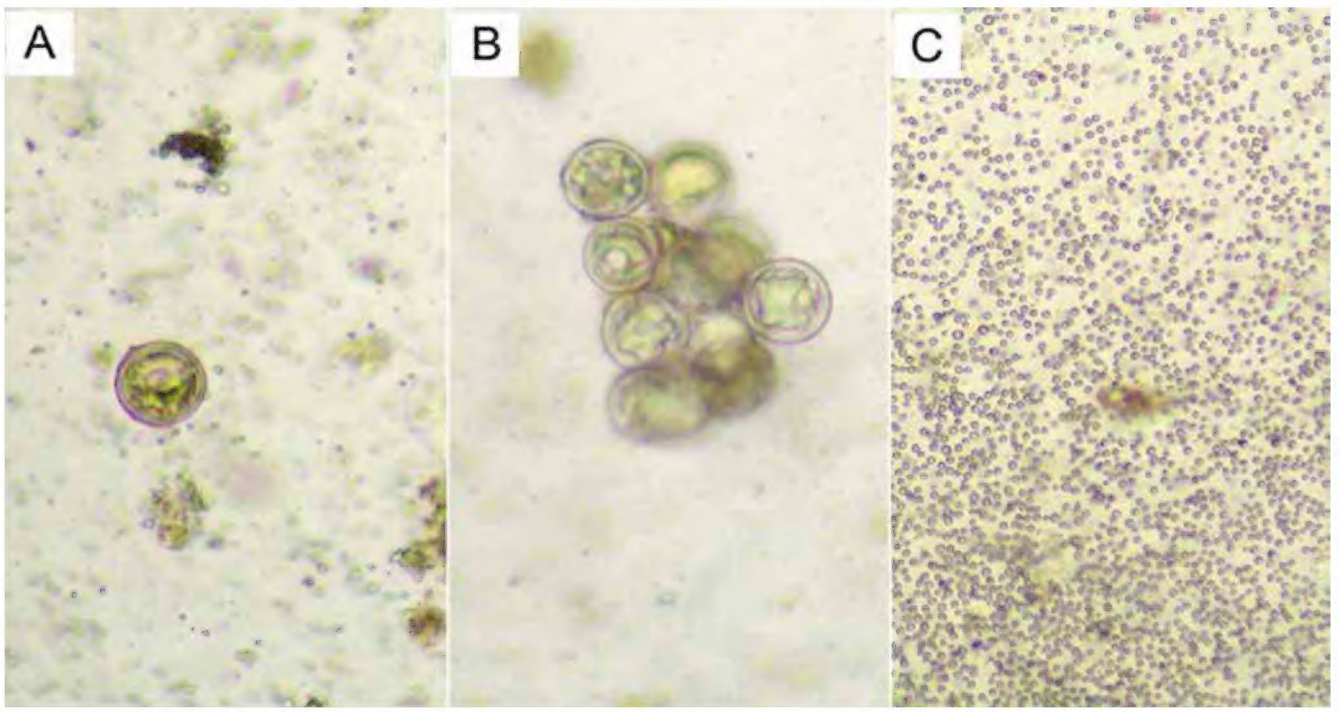

Figure 2. Combined fungal-viral infection in *Lymantria dispar* larvae: A – single azygospores of *E. maimaiga* and low concentration of *Ld*MNPV polyhedra (22.06.2022); B – sporadic azygospores of *E. maimaiga* and very low concentration of *Ld*MNPV polyhedra (22.06.2022); C – high concentration of *Ld*MNPV polyhedra without *E. maimaiga* azygospores (14.07.2022) (250× magnification)

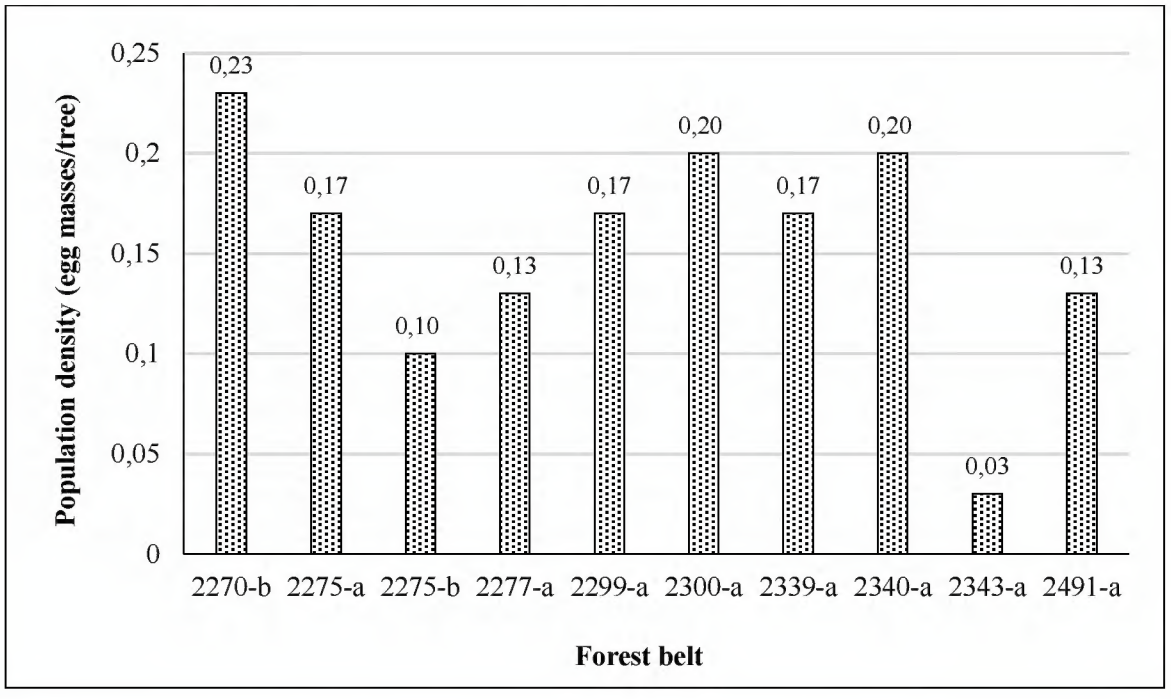

Figure 3. Population density of *L. dispar* at the territory of SHE Balchik

than 50 and more than 15000 egg masses/ha (Hajek, 1999). According to the author, there is still no clear relationship between the prevalence of *E. maimaiga* infection and the abundance of *L. dispar*. The high efficiency of the pathogen could be due both to the presence of high concentrations of azygospores in the epizootic sites, and to favourable weather conditions – frequent and abundant wetting of the leaves, contributing to the enhancement of the release of *E. maimaiga* conidia into the environment.

The combined effect of *E. maimaiga* and *Ld*MNPV on *L. dispar* population was investigated in North America, where the virus was reported in 1909, most likely accidentally introduced with the host (Hajek, 1999). *Ld*MNPV is relatively slow-acting, and *L. dispar* populations usually reach unacceptably high densities before the virus causes mass mortality. The author notes that *E. maimaiga* and *Ld*MNPV can infect individual larvae of *L. dispar*, but the percentage of carcasses in which both pathogens reproduce is negligible, mostly 0.8%, except for one case of 44%.

Laboratory studies at 20°C showed that most *L. dispar* larvae co-infected with *E. maimaiga* and *Ld*MNPV died within 5–7 days, with the carcasses carrying only *E. maimaiga* spores and no viral occlusal bodies (Hajek, 1999). Larvae that were not killed by *E. maimaiga* died from *Ld*MNPV infections at the expected time (14 days). In addition, coinfection with *E. maimaiga* exposure, after infection of larvae with *Ld*MNPV for 10 days, shortened the time before death, with co-infected larvae dying 1–2 days earlier than positive controls infected with *Ld*MNPV alone. Therefore, in co-infection or in cases where the *E. maimaiga* infection precedes the *Ld*MNPV infection, *E. maimaiga* dominates, killing the co-infected insects, whereas in larvae infected first with *Ld*MNPV and subsequently with *E. maimaiga*, death occurs earlier and viral reproduction decreases, resulting in less deposition of viral inoculum in the environment.

The impact of the introduced pathogen *E. maimaiga* on *Ld*MNPV and other native biotic limiting factors, incl. parasitoids is important for the biological control of *L. dispar*. The influence of *E. maimaiga* on survival of Tachinidae (Diptera) parasitoids associated with late-instar host larvae (*Compsilura concinnata*, *Exorista larvarum*, *Senometopia separata*, *Senometopia excisa*, *Drino incospiqua*, *Zenilia libatrix*) was studied in Bulgaria and Serbia (Georgiev et al., 2013b; Tabaković-Tošić et al., 2014). Despite the lack of direct evidence of infection of parasitoid larvae and pupae by the pathogen, the mortality rate is extremely high (86.5% in Bulgaria and 97.1% in Serbia), which is related to the deterioration of the conditions for the nutrition of the parasitoids during their co-development with *E. maimaiga* in the host larvae.

In conclusion, it should be noted that the introduction of *E. maimaiga* in 2021 in the field protective forest belts at the territory of SHE Balchik resulted in population collapse of the pest in 2022. In addition, the observed co-infection of *E. maimaiga* and *Ld*MNPV in the gypsy moth population in the region not only increases the existing knowledge about the impact of the fungal pathogen, but also contributes to clarifying its relationships with the native biotic agents of the pest in Bulgaria.

Acknowledgments

This research was conducted in connection with the implementation of the National Scientific Program (NSP) 'Protection of the environment and reduction of the risk of adverse phenomena and natural disasters', approved by Decision of the

Ministry of the Interior No. 577/17.08.2018 and financed by Ministry of Education and Culture (Agreement No. D01-271/09.12.2022), and with the implementation of the project 'Deterioration of the health status of the field protective forest belts in North-Eastern Bulgaria and opportunities for improvement and reconstruction', financed by the National Fund of Science (No. KP-06-H66/9/13.12.2022).

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